Iranian Journal of Veterinary Science and Technology
Vol. 5, No. 2, 2013, 45-52

IJVST

Seroprevalence of *Theileria equi* and *Babesia caballi* infection in Turkoman breed horses in Iran

Vali Abedi[1], Gholamreza Razmi[2,*], Hesam Seifi[3] and Abolghasem Naghibi[2]

[1]Student of Veterinary Parasitology (Ph.D),
Department of Pathobiology, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran
[3]Department of Clinical Sciences, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran

Received: April 6, 2013   Accepted: July 9, 2013

Abstract

Equine babesiosis is a hemoprotozoan tickborne disease with worldwide distribution and caused by *Theileria equi* and *Babesia caballi.* This study was conducted to determine the seroprevalence of *T. equi* and *B. caballi* infection in Turkoman breed horses in North Khorasan Province of Iran. Blood samples were collected from 100 apparently healthy horses and examined by microscopy and indirect immunofluorescent antibody test. *T. equi* was microscopically detected in five blood smears. Antibodies against *T. equi*, *B. caballi* and dual infection were found in 48 (48%), 2 (2%) and 3 (3%) serum samples, respectively. No significant difference was observed between the seroprevalence of piroplasm infection with risk factors such as age, gender and activity in horses. This is the first report of detection of *T. equi* and *B. caballi* infection using IFAT in Iran. It was concluded that the seroprevalence of *T. equi* infection is higher than *B. caballi* infection in Turkoman breed horse in Iran.

Keywords: *Theileria equi*, *Babesia caballi*, Indirect immunofluorescent antibody test, Turkoman breed horse

*Corresponding author:Gholamreza Razmi
Email:razmi@ferdowsi.um.ac.ir
Tel: +98 51 38788944
Fax: +98 51 38763853
P.O.: 91775-1793

Introduction

Equine piroplasmosis is an important tickborne hemoprotozoan disease in horses, mules, donkeys and zebras. It is caused by *Theileria equi* and *Babesia caballi* (Mehlhorn and Schein, 1998, Sellon and Long, 2007).

The disease is characterized by fever, anemia, icterus, hepatosplenomegaly, bilirubinuria and hemoglobinuria (de Waal, 1992). Most of the clinical cases are caused by *T. equi* while infection with *B. caballi* tends to be inapparent (Friedhoff *et al*., 1990). Carrier horses may show clinical disease under stress condition (Hailat *et al*., 1997).

Detection of *Theileria* and *Babesia* parasites can be done by different methods. Microscopical examination of stained blood smear is a traditional diagnostic method of piroplasm detection in acute phase of disease, but it is almost impossible to detect parasites in carrier horses with low parasitemia (Bose *et al*., 1995).

Several serological techniques have been developed to detect antibodies against blood parasites in subclinical or chronic forms of disease. The indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are considered to more sensitive than other serological methods and it is recommended for detection of *T.equi* and *B.caballi* infection in international trade of horses (OIE, 2008). *B. caballi* and *T. equi* were reported in horses from Iran (Aslani, 2000, Seifi *et al*., 2000) but there is not any information about epidemiology of piroplasm infection in horses of Iran. The aim of study was to determine the seroprevalence of the *T. equi* and *B. caballi* infection in Turkoman breed horses in Iran and evaluating the relationship between risk factors with seropositivity.

Matrials and methods

Field study area

This study was carried out in North Khorasan Province located in northeastern of Iran between 36°37′-38°17′ N latitudes and 55°53′-58°20′ E longitudes with an area of more than 28400 km^2. It is situated next to the northeastern border of Iran, level with the southern Caspian sea and south of Turkmenistan. This province is a mountainous area and receives about 250 mm of rainfall annually. Turkoman horses breed are reared by turkmen tribes in the northern areas of the province.

Sampling

One hundred horses were randomly selected form 14 villages from June to August 2011. Data of each horse including age, gender, activity and any grazing in pasture were recorded. Blood samples were taken from jugular vein and placed into two sterile tubes with and without EDTA. The blood tubes were kept in cooled condition and immediately transmitted to the laboratory. The sera were separated by centrifugation at 4000 rpm for 10 min and stored at -20˚C until the serological examination.

Examination of blood smears

The boold smears were prepared from blood samples in EDTA tubes. The smears were fixed in methanol and stained in 10% Giemsa solution in phosphate buffered saline (PBS) pH 7.2. The slides were examined with an oil immersion lens at total magnification of ×1000. In order to identify *Theileria equi* and *Babesia caballi* species, the full length of intraerythrocytic mature piroplasm organism was measured by a graded ocular microscopy at magnification of ×1000 (Soulsby, 1982). Parasitemia was assessed by counting the number of infected red blood cells on examination of 50 microscopic fields (approximately 50000 cells). The infected number was then expressed as percentage.

Serology

IFAT was done according to manufacturer's instructions for *T. equi* antibodies (Fuller Laboratories, Fullerton, Callifornia, USA) and for *B. cabali* antibodies (MegaScreen, Horbranz, Austria). Sera will

considered as positive if the parasites showed fluorescence reaction at dilution of 1:80.

Statistical analysis

Any relationship between infection rate and risk Factors such as age, gender and activity were analyzed by Chi-square test. Significant associations were statistically identified when a p-value of less than 0.05 was observed.

Results

T. equi infection were microscopically detected in 5 (5%) of blood smears with low parasitemia (approximately 0.001-0.003%). *T. equi* is smaller than *B. caballi*, about 2 μ and frequently form maltese cross (Fig.1). Serological examination revealed that 51 (51%), 2 (2%) and 3 (3%) had antibodies against *T. equi*, *B. caballi* and mixed infection, respectively (p<0.05) (Table 1).

The frequency of antibodies against *T. equi* in different groups of ages, activity and gender in horses were not statistically different (p>0.05) (Table 2).

Table 1. Results of microscopical and serological examination of *B.caballi* and *T.equi* infection in one hundred horses in North Khorasan Province

species	Microscopic examination No(%)	Serology No(%)
B.caballi	0	2
T.equi	5	48
Mixed infectiom	0	3
Total	5	53

Table 2. Frequency of antibodies against *T. equi* and *B. caballi* infections by different risk factors in 100 turkoman breed horses (No*: No per group).

		T. equi		*B. caballi*		Mixed infection		Total	
	No*	No	%	No	%	No	%	No	%
Age (year)									
<1	7	2	28.57	0	0	0	0	2	28.57
1-2	24	8	33.33	0	0	2	8.33	10	41.66
3-5	31	14	45.16	2	6.45	0	0	16	51.61
5<	38	24	63.15	0	0	1	2.63	25	64.1
Activity									
Stud	33	20	60.6	0	0	1	3.03	21	63.63
Racehorse	67	28	41.79	2	2.98	2	2.98	32	47.76
Gender									
Male	21	8	38.09	1	4.76	0	0	9	42.85
Female	79	40	56.63	1	1.26	3	3.79	44	55.69

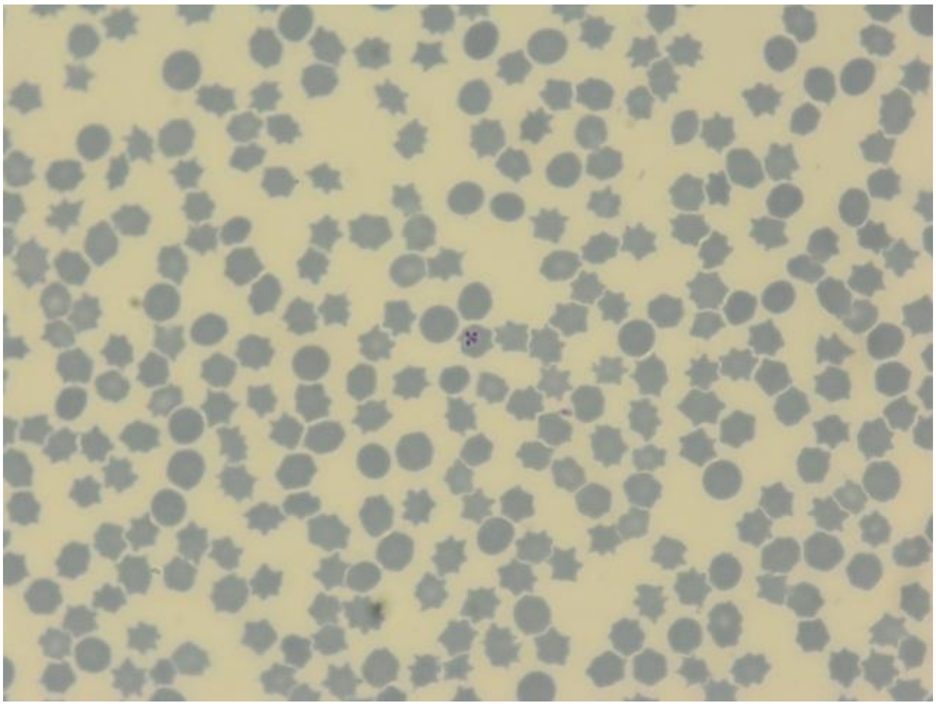


Figure 1. Maltese-cross form of *T.equi* (at magnification of ×1000.)

Disscusion

In this study, *T. equi* infection was microscopically detected in 5 (5%) blood samples with low parasitemia, while 53% of horses were found seropositive for piroplasms infection using IFAT. The results confirmed low sensitivity of the microscopical examination in comparison wiht serological methods. Some studies were shown the high sensitivity and specificity of IFAT to differentiate *T. equi* and *B. caballi* in horses (Ogunremi *et al*., 2007; Ogunremi *et al*., 2008). In the present study, Antibodies against *T. equi*, *B. caballi* and mixed infection were detected in 48 (48%), 2 (2%) and 3 (3%) of horses, respectively. Seropositivity rates of *T. equi* and *B. caballi* infections were reported 10.4 and 7.5% in Saudi Arabia (Alanazi *et al*., 2012), 77.1 and 11.4% in Kuwait (Donelly *et al*., 1980), 97.7 and 40.9% in Oman (Donelly *et al*., 1980), 12.8 and 9.6% in Turkey (Karatepe *et al*., 2009) by IFAT and 16.21 and 0.83% in Turkey (Sevinc *et al*., 2008), 81.11 and 18.88% in Iraq (Al-saad, 2009), 32.45 and 15.2% in United Arab Emirates (Jaffer *et al*., 2010), 14.6 and 0% in Jordan (Abutarbush *et al*., 2012), by ELISA, respectively. The serological studies indicated that *T. equi* infection is more prevalent than *B. caballi* in Middle East.

The seropositivity rate of *T. equi* infection in different age groups of horses were non significant. The result was similar to the studies that done in Turkey (Acici *et al*., 2008; Karatepe *et al*., 2009), Italy (Moretti *et al*., 2010), Israel (Shkap *et al*., 1998, Steinman *et al*., 2012), Switzerland (Sigg *et al*., 2010) and Venezuela (Mujica *et al*., 2011) and on the contrary other studies that done in Mongolia (Ruegg *et al*., 2007) and Greece (Kouam *et al*., 2010).

A mare with her foal (one month old) were also seropositive against *T. equi* infection. The foal was healthy without any clinical signs. It seems the antibodies against *T. equi* were transfererd to foal via colostrum. The antibodies in colostrum usually decrease after 63-77 days post foaling (Kumar *et al*., 2008).

The difference of serporevalence in male and female horses was not statistically significant. This finding agrees with the results of the studies in Turkey (Karatepe *et al*.,

2009), Israel (Steinman *et al*., 2012), Switzerland (Sigg *et al*., 2010), Greece(Kouam *et al*., 2010), Venezuela (Mujica *et al*., 2011) and Trinidad (Asgarali *et al*., 2007) but disagreed with other studies that done in Israel (Shkap *et al*., 1998) and Mongolia (Ruegg *et al*., 2007)

Non-significant difference was obtained between the seroprevalence of piroplasm infections with different activity in horses. The turkoman horses with different activity graze in the pasture during spring and summer and chances of tick biting and piroplasm infection is equal. Some studies were shown that the grazing and different kinds of horse mangment could be pronounced risk factors for equine piroplasmosis (Moretti et al., 2010, Kouam , et al 2010, Sevinc *et al*., 2008). To the knowledge of the authors, this is the first study of seroprevalence of piroplasmosis in Turkoman horses in Iran. The results showed that *T. equi* infection is more prevalent than *B. caballi* infection.

Acknowledgements

We thank Dr. Abdoljalil ghiadi the manager of Turkoman horse breeding company (THBC) for his hospitality and aid for sampling, Mohammad Abedi and Dr. Ali Sarani for their aid for sampling and data collection.

References

Abutarbush, S.M., Alqawasmeh, D.M., Mukbel, R.M., Al-Majali, A.M. (2012) Equine babesiosis: seroprevalence, risk factors and comparison of different diagnostic methods in Jordan. *Transboundary Emerging Diseases* **59**, 72-78.

Acici, M., Umur, S., Guvenc, T., Arslan, H.H., Kurt, M. (2008) Seroprevalence of equine babesiosis in the Black Sea region of Turkey. *Parasitology International* **57**, 198-200.

Alanazi, A.D., Alyousif, M.S., Hassieb, M.M. (2012) Seroprevalence study on *Theileria equi* and *Babesia caballi* in horses from central province of Saudi Arabia. *Journal of Parasitology* **98**, 1015–1017.

Al-Saad, K.M. (2009) Acute babesiosis in foals. *Journal of Animal and Veterinary Advances* **8**, 2585-2589.

Asgarali, Z., Coombs, D.K., Mohammed, F., Campbell, M.D., Caesar, E. (2007) A serological study of *Babesia caballi* and *Theileria equi* in Thoroughbreds in Trinidad. *Veterinary Parasitology* **144**, 167-171.

Aslani, M.R. (2000) A case report of *Babesia caballi* infection in a foal. *Journal of Applied Animal Research* **7**, 253-256.

Bose, R., Jorgensen, W.K., Dalgliesh, R.J., Friedhoff, K.T., de Vos, A.J. (1995) Current state and future trends in the diagnosis of babesiosis. *Veterinary Parasitology* **57**, 61-74.

de Waal, D.T. (1992) Equine piroplasmosis: a review. *British Veterinary Journal* **148**, 6-14.

Donnelly, J., Joyner, L.P., Graham-Jones, O., Ellis, C.P. (1980) A comparison of the complement fixation and immunofluorrescent antibody tests in a survey of the prevalence of *Babesia equi* and *Babesia caballi* in horses in sultanate of Oman. *Tropical Animal Health and Production* **12**, 50-60.

Donnely J., Joyner, L.P., Frank, C. (1980) Quantitative epidemiological studies on the prevalence of babesiosis in horses in Kuwait. *Tropical Animal Health and Production* **12**, 253-258.

Friedhoff, K.T., Soule C. (1996) An account on equine babesioses. *Revue Scientifique et Technique Office International des Epizooties* **15**, 1191-1201.

Friedhoff, K.T., Tenter, A.M., Muller, I. (1990) Haemoparasites of equines: impact on international trade of horses. *Revue Scientifique et Technique Office International des Epizooties* **9**, 1187-1194.

Hailat, N.Q., Lafi, S.Q., A1-Darraji, A.M., A1-Ani, F.K. (1997) Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. *Veterinary Parasitology* **69**, 1-8.

Jaffer, O., Abdishakur, F., Hakimuddin, F., Riya, A., Wernery, U., Schuster, R.K. (2010) A comparative study of serological tests and PCR for the diagnosis of equine piroplasmosis. *Parasitology Research* **106**, 709-713.

Karatepe, B., Karatepe, M., Cakmak, A., Karaer, Z., Ergun, G. (2009) Investigation of seroprevalence of *Theileria equi* and *Babesia caballi* in horses in Nigde province, Turkey. *Tropical Animal Health and Production* **41**, 109-113.

Kouam, M.K., Kantzoura, V., Gajadhar, A.A., Theis, J.H., Papadopoulos, E., Theodoropoulos, G. (2010) Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. *Veterinary Parasitology* **169**, 273-278.

Kumar, S., Kumar, R., Gupta, A.K., Dwivedi, S.K. (2008) Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. *Veterinary Parasitology* **151**, 80-85.

Mehlhorn, H., Schein, E. (1998) Redescription of *Babesia equi* Laveran. 1901 as *Theileria equi*. *Parasitology Research* **84**, 467-475.

Moretti, A., Mangili, V., Salvatori, R., Maresca, C., Scoccia, E., Torina, A., Moretta, I., Gabrielli, S., Tampieri, M.P., Pietrobelli, M. (2010) Prevalence and diagnosis of *Babesia* and *Theileria* infections in horses in Italy: A preliminary study. *The Veterinary Journal* **184**, 346-350.

Mujica, F.F., Perrone, T., Forlano, M., Coronado, A., Melendez, R.D., Barrios, N., Alvarez, R., Granda, F. (2011) Serological prevalence of *Babesia caballi* and *Theileria equi* in horses of Lara State, Venezuela. *Veterinary Parasitology* **178**, 180-183.

OIE (2008) Equine piroplasmosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.05.08_EQUINE_PIROPLASMOSIS.pdf (Chapter 2.5.8).

Ogunremi, O., Georgiadis, M.P., Halbrt, G., Benjamin, J., Pfister, K., Lopez-Rebollar, L. (2007) Validation of the indirect fluorescent antibody and the complement fixation tests for diagnosis of *Theileria equi*. *Veterinary Parasitology* **148**, 102-108.

Ogunremi, O., Halbert, G., Mainar-Jaime, R., Benjamin, J., Pfister, K., Lopez-lebollar, L., Georgiadis, M.P. (2008) Accuracy of an indirect fluorescent-antibody test and of a complement fixation test for the diagnosis of *Babesia caballi* in field samples from horses. *Preventive Veterinary Medicine* **83**, 41-51.

Ruegg, S.R., Torgerson, P., Deplazes, P., Mathis, A. (2007) Age-dependent dynamics of *Theileria equi* and *Babesia caballi* infections in southwest Mongolia based on IFAT and/or PCR prevalence data from domestic horses and ticks. *Parasitology* **134**, 939-947.

Seifi, H.A., Mohri, M., Sardari, K. (2000) A mixed infection of *Babesia equi* and *Babesia caballi* in a racing colt: a report from Iran. *Journal of Equine Veterinary Science* **20**, 858-860.

Sellon, D.B., Long, M.T. (2007) Equine infectious diseases, 1st edn., Saunders, Missouri. pp 465-473.

Sevinc, F., Maden, M., Kumas, C., Sevinc, M., Ekici, O.D. (2008) A comparative study on the prevalence of *Theileria equi* and *Babesia caballi* infections in horse sub-populations in Turkey. *Veterinary Parasitology* **156**, 173-177.

Shkap, V., Cohen, I., Leibovitz, B., Savitsky, Pipano, E., Avni, G., Shofer, S., Giger,

U., Kappmeyer, L., Knowles, D. (1998) Seroprevalence of *Babesia equi* among horses in Israel using competitive inhibition ELISA and IFA assays. *Veterinary Parasitology* **76**, 251-259.

Sigg, L., Gerber, V., Gottstein, B., Doherr, M.G., Frey, C.F. (2010) Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population. *Parasitology International* **59**, 313-317.

Soulsby, E.J.L. (1982) Helminths, arthropods and protozoa of domesticated animals, 7th edn., Bailliere Tindall, London. pp 712-715.

Steinman, A., Zimmerman, T., Klement, E., Lensky, I.M., Berlin, D., Gottlieb, Y., Baneth, G. (2012) Demographic and environmental risk factors for infection by *Theileria equi* in 590 horses in Israel. *Veterinary Parasitology* **187**, 558-562.

Iranian Journal of Veterinary Science and Technology
Vol. 5, No. 2, 2013, 45-52

IJVST

بررسی شیوع سرمی ابتلا به *تیلریا اکویی* و *بابزیا کابالی* در اسب های نژاد ترکمن در ایران

ولی عابدی[۱]، غلامرضا رزمی[۲]، حسام الدین سیفی[۳]، ابوالقاسم نقیبی[۲]

[۱] *دانشجوی دکتری انگل شناسی دامپزشکی، گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران*
[۲] *گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران*
[۳] *گروه علوم درمانگاهی ، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران*

دریافت مقاله : ۱۳۹۲/۰۱/۱۷ پذیرش نهایی: ۱۳۹۲/۰۴/۱۸

چکیده

پیروپلاسموز اسبی بیماری تک یاخته ای خونی منتقله از کنه با انتشار جهانی است و عامل آن *تیلریا اکویی* و *بابزیا کابالی* می باشد. این مطالعه به منظور تعیین شیوع سرمی ابتلا به آلودگی *تیلریا اکویی* و *بابزیا کابالی* در اسب های نژاد ترکمن در استان خراسان شمالی صورت گرفت. ارتباط بین ابتلا به عفونت پیروپلاسمی و فاکتورهای خطر از قبیل سن، جنس و نوع فعالیت نیز مورد بررسی قرار گرفت. نمونه های خون از تعداد ۱۰۰ راس اسب ترکمن به ظاهر سالم اخذ گردید. تک یاخته *تیلریا اکویی* در ۵ گسترش خونی مشاهده شد. آنتی بادی علیه *تیلریا اکویی*، *بابزیا کابالی* و آلودگی مخلوط دو گونه به ترتیب در ۴۸ نمونه (۴۸٪) ، ۲(۲٪) و ۳ (۳٪) نمونه تعیین گردید. بین فاکتورهای خطر شامل سن، جنس و نوع فعالیت و میزان سرم مثبت برعلیه عفونت، تفاوت معنی داری مشاهده نگردید. این اولین گزارش در مورد بررسی ابتلا به *تیلریا اکویی* و *بابزیا کابالی* در ایران با استفاده از روش ایمنوفلوئورسانس غیر مستقیم می باشد. این مطالعه نشان می دهد که میزان شیوع آلودگی *تیلریا اکویی* بیشتر از شیوع آلودگی *بابزیا کابالی* در اسب های نژاد ترکمن در ایران می باشد.

واژگان کلیدی: *تیلریا اکویی*، *بابزیا کابالی*، ایمنوفلوئورسانس غیر مستقیم، اسب نژاد ترکمن